Acta Scientific Agriculture (ISSN: 2581-365X)

Volume 2 Issue 6 June 2018

Research Article

Effect of Chemical and Biological Treatment for the Control of Seed-Borne Mycoflora of Barley (*Hordeum vulgare* L.)

Lukman Ahamad* and Razia K Zaidi

Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh, India

***Corresponding Author:** Lukman Ahamad, Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh, India.

Received: April 10, 2018; **Published**: May 09, 2018

Abstract

Dry seed inspection showed that seed abnormalities and conidial fructifications were observed under naked eye and stereobinocular microscope. Seed health techniques confirmed the most frequently isolated fungus was *Alternaria alternata* followed by *Rhizopus stolonifer, Mucor* spp., *Fusarium moniliforme* and *Aspergillus flavus* determined by seed plating on both standard blotter method and agar plate method respectively. For the control of seed-borne fungi fungicides viz. Mancozeb, Carbendazim and Mancozeb plus Carbendazim mixture as well as bioagents *Trichoderma viride, Trichoderma harzianum* and *Aspergillus niger* were tested through poisoned food and dual culture technique respectively. The results indicated that different concentrations of fungicides and bioagents were found significantly effective in percent growth inhibition of *A. alternata, R. stolonifer, Mucor* spp., *F. moniliforme* and *A. flavus*. Among fungicides, Mancozeb plus Carbendazim mixture was found highest growth inhibition at 300 ppm concentration followed by Mancozeb and Carbendazim alone over control. However, in biocontrol agent's highest percent growth inhibition was observed in *T. viride* followed by *T. harzianum* and *A. niger* as compared to control. Further seed priming with Mancozeb plus Carbendazim and *T. viride* was also found to be effective in eliminating seed-borne fungi and results percent germination increase in both agar plate method and blotter method over control. During pot experiment treated seeds were found significantly effective in increase plant growth and chlorophyll content over control plants.

Keywords: Biocontrol Agents; Fungicides; Seed Inspection; Seed-Borne Fungi; Seed Treatment

Introduction

Seed-borne diseases are major threat to crop production and yield in almost all cultivated crops across the globe by causing pre and post-infections. These have been found to affect the growth and productivity of crop plants [1]. Seeds provide natural substrate for the growth of fungi gets associated with externally or internally or both to the seeds. Fungi associated with seeds as contaminant can cause seed abnormalities, poor germination as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection [2,3]. For better crop improvement there is need to produce healthy and disease free seeds. A number of chemical agents are used to control these pathogens but management of diseases using biocontrol agents is crucial for the future crop cultivation. A large number of fungicides are being used in the form of dusting, slurry and soaking treatment [4]. Seed dressing fungicides have long been applied to cereal seeds to prevent seed decay, damping-off and seedling blight, and seed-borne fungi [5]. Even though effective and efficient control of seed-borne fungi can be achieved through application of synthetic chemical fungicides, the same cannot be applied to the grains due to pesticide toxicity [6]. It is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms [7]. Seed treatment is the safest and cheapest practice to control seed-borne fungal pathogens [8]. In relation to this fungicides and biological control agents used as seed treatments or seed priming. Biocontrol agents are the microorganisms that protect seeds and seedlings from numerous seed-borne fungi [9,10]. The pursuit of alternatives to chemical pesticides and an increasing interest in "organic" production methods come into the force to increase scientific development of biological control agents over the years. In this regard, seed health testing and management need to be understood in light of the general evolution of the seed sector. Besides, increasing crop productivity seed health issues are extremely important in international seed trade, conservation, and utilization of plant genetic resources, which is vital for global food security. So, with this view, the present investigation was undertaken to evaluate fungicides and biocontrol agents that could be effective in the development of new tools for the management of seed-borne fungi to the plants of economic importance.

Materials and Methods

Seeds collection

Seed of barley cv. Narendra barley-2 were collected in gunny bag from the agricultural farm of the Aligarh district of (UP) India. Seed sample were stored at room temperature 28oC till processing and examined for the seed-borne mycoflora according to the international rules for seed testing [11].

Dry seed examination

Inspection of dry seeds can be applied to detect seed-borne pathogens present in the seed. It may cause discoloration of seed coat or changes in the seed size and shape. Four hundred seeds were examined in a transparent petri-plate with unaided eye. Seeds with mechanical damage, abnormalities, discoloration, smut balls and other fungal bodies were observed under stereoscopic microscope and percentage recorded. Soil clods, sand, stone pieces were also separated. As per 1STA rules (ISTA, 1993), these impurities are considered inert matter.

Standard blotter method

The standard blotter method was developed later it included in the International seed Testing Association (ISTA) rules of 1966 [12,13]. In this method, ten seeds were plated in sterilized petri-plate (9 cm diameter) containing three moistened blotter paper by dropping off extra water. The seeds were plated at equidistance in the petri-plate with the help of sterilized forceps. There were three replications of each treatment. The plates were incubated at 28 ± 2oC for alternate periods of 12 hours light and 12 hours darkness for 7 days. The plates were removed on the eighth day. In this way, three hundred seeds were observed, and fungi developed on seeds were examined under 40x-50x magnification of the stereo-binocular microscope. Different seed-borne fungi were identified on the basis of form, length, conidiophores arrangement, size and septation as well as chain formation of conidia [14].

Agar-plate method

Agar plate method is another popular method for the detection of seed-bone fungi, in which seeds were plated on agar media. The seeds were plated at equidistance in the petri-plate (9 cm diameter) with the help of sterilized forceps. Plated seeds were then incubated at 28 ± 2°C for 7 days with cycles of 12 hours of light and 12 hours of darkness. In this way, three hundred seeds were observed, and fungi developed on seeds were examined and identified under 40x-50x magnification of the stereo-binocular microscope [14].

Poison food technique

Antifungal activity of fungicides viz., Mancozeb 75% WP (Dithane M-45), Carbendazim 50% WP and Mixture of Carbendazim 12 + Mancozeb 63% WP were tested against mycelial growth of dominant seed-borne fungi [15]. Each fungicide was applied @ 100, 200, 300 ppm concentration in the test and mixed well with autoclaved agar medium. After that 5 mm diameter agar disk of *Alternaria alternata, Rhizopus stolonifer, Mucor* spp., *Fusarium moniliforme* and *Aspergillus flavus* were cut from active culture by using sterile cork borer and placed in the center of petri-plates containing the different concentration of fungicide. There were three replicates of each treatment. Petri-plates without fungicidal treatment were served as control. The plates were then incubated at 28 ± 2oC for 7 days. The percent inhibition of mycelial growth was calculated as per formula [16]. The fungicide which was found effective in the laboratory conditions were applied for seed treatment in both standard blotter and agar plate methods.

Dual culture technique

Dual culture technique consists of growing the test organism and pathogenic organism on the same plate [17]. Agar media 20 ml of melted cooled (45 - 50°C) was poured in each petri-plate and allowed to solidify. A 5 mm mycelial disc cut from the margin of the actively growing colonies of pathogenic culture (*A. alternata, R. stolonifer, Mucor* spp., *F. moniliforme* and *A. flavus*) was placed near the periphery on one side of the agar plate. Another disc of 5 mm of test organism (*Trichoderma viride, Trichoderma harzianum, Aspergillus niger*) was also placed on the other side of the same plate opposite to the first disc at an angle of 180°C. There were three replicates of each treatment. Petri-plates without test organisms served as control. Inoculated petri-plates were incubated at 28 ± 2°C for 7 days and colony growth inhibition was calculated. The biocontrol agents found effective in the laboratory conditions were evaluated for seed treatment in both agar plate and blotter methods.

$$\text{Mycelial growth inhibition (\%)} = \frac{dc - dt}{dc} \times 100$$

Where dc is the average colony diameter of fungal growth in control and dt is the average diameter in treatment.

Preparation of conidial suspension

A conidia suspension of *T. viride* was prepared from the 7-day old culture of the isolate on agar media. 1g mycelial mat was flooded with 10 ml of sterilized water and shaken for a few minutes. The resulting suspension was filtered through muslin cloth. After that, the conidial concentration was determined by using haemocytometer. The spore suspension was adjusted to 10^6 conidia/ml using sterilized water.

Seed priming

Seed treated with Mancozeb + Carbendazim mixture and *T. viride* (10^6 conidia/ml) (as they found to be effective in poison food technique) three hundred moderately infected seeds were placed in 150 ml flasks with 300 ppm concentration of fungicide and with prepared condial suspension of *T. viride*. The flasks were plugged and shaken for 20 minutes on the mechanical shaker so as to get the uniform distribution on the seed surface. The seeds after fungicidal and biocontrol treatment were removed from flasks and dried on blotter sheet separately for 2 hours. After that coated seeds were placed on sterilized petri-plates containing moist blotter paper and on agar media and then incubated at 28 ± 2°C for 7 days. There were 3 replicates of each treatment. Plated seeds without treatment were served as control. After incubation, fungi growing out from the seeds were examined and relative frequency of seed-borne fungi, percent germination was calculated by following given formula.

$$\text{Relative frequency} = \frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds used}} \times 100$$

$$\text{\% germination} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Pot experiment

During pot experiment coated seeds sown in 2 cm deep in earthen pots (25 cm diameter) filled with sterilized soil. After 110 days of sowing, data on plant length (cm), plant fresh weight (g), plant dry weight (g), number of grains/spike, number of spike/plant, and chlorophyll content [18] were recorded.

Data Analysis

Data was analyzed by one-way analysis of variance (ANOVA) by using R software (R Development Core Team 2011). Least significant difference (L.S.D) and Duncan's multiple range tests were calculated at $P \leq 0.05$ to test for significant differences.

Results

Inspection of Dry Seeds

In dry seed inspection, 400 seeds were examined with naked eye and stereobinocular microscope (40x-50x) for contamination and other apparent disorders. The results indicated that seeds were deformed, damaged, discoloured, contaminated with inert matter and infected with condia as well as mycelial fragments (Table 1).

S. NO.	Observed Characteristics	Remarks
1.	Color of pericarp	Light yellow
2.	Discoloration and blemishes	4.5%
3.	Mycelial bits and conidia	1.5%
4.	Damaged seeds	12.75%
5.	Deformed seeds	1.5%
6.	Broken seeds	5.25%
7.	Malformed seeds	3.75%
8.	Inert matter	1.25%

Table 1: Inspection of dry seeds of barley variety-Narendra barley-2 (observation based on 400 seeds)

Isolation of Seed-Borne Mycoflora

Seed borne fungi isolated from standard blotter method was *A. alternata* (65.5%), *R. stolonifer* (51.8%), *Mucor* spp. (47.24%), F. moniliforme (41.8%), A. flavus (34.65%), A. niger (31.5%), *Penicillium* spp. (27.03%), *D. australiensis* (18.5%), *C. lunata* (16.71%), *Cladosporium* spp. (9.56%), *Stemphylium* spp. (4.51%) and *Ulocladium* spp. (2.04%). However, in agar plate method, seed-borne fungi isolated and identified was *A. alternata* (41.5%), *R. stolonifer* (24.31%), *Mucor* spp. (18.4%), *F. moniliforme* (30.75%), *A. flavus* (16.8%), *A. niger* (36.25%), *Penicillium* spp. (12.45%), *D. australiensis* (27.51%) and *C. lunata* (24.6%). Percent germination of seeds was observed 42 in standard blotter method and 46 in agar plate method (Table 4, Figure 1a-1c).

Fungicides	Concentration (ppm)	Percent growth inhibition				
		Alternaria alternata	*Rhizopus* spp.	*Mucor* spp.	*Fusarium moniliformae*	*Aspergillus flavus*
Mancozeb	100	76.66 bcd	72.02 cde	69.64 cd	68.86 cde	65.77 cde
	200	77.80 bcd	74.10 bcd	73.33 bc	70.83 cd	68.85 c
	300	79.61abc	76.42 abc	74.78 bc	72.97 bc	69.52 bc
Carbendazim	100	72.92 d	67.33 e	66.25 d	64.76 e	62.55 e
	200	76.04 cd	70.04 de	67.97 d	67.08 de	64.40 de
	300	77.32 bcd	71.84 cde	70.61 cd	69.15 cde	67.33 cd
Mancozeb + Carbendazim	100	80.35 abc	76.04 bc	74.16 bc	73.27 bc	70.17 bc
	200	81.78 ab	78.21 ab	77.97 ab	76.25 ab	73.33 ab
	300	83.21 a	81.25 a	80.00 a	79.34 a	75.04 a
C.D. P ≤ 0.05		**4.62**	**4.65**	**4.88**	**4.56**	**4.09**

Table 2: In vitro Evaluation of fungicides against seed-borne fungi by poison food technique.

Each value is the average of three replicates

*Values in a column followed by the different letters are significantly different at P ≤ 0.05 using Duncan's multiple range test

*C.D: Critical Difference

Effect of Fungicides on the Growth of Dominant Seed Borne Fungi

Fungicides were tested against seed-borne fungi was Mancozeb, Carbendazim and Mixture of Mancozeb and Carbendazim at 100, 200, 300 ppm concentration. All the doses of fungicides were found significantly effective in mycelial growth inhibition against dominant fungi isolated from barley seeds. Among them, Mancozeb+ Carbendazim mixture at 300 ppm concentration was found most effective against *A. alternata, R. stolonifer, Mucor* spp., *F. moniliforme* and *A. flavus* over control (Table 2).

Evaluation of Biocontrol Agents Against Seed-Borne Fungi

The antagonistic effects of three biocontrol agents viz., *Trichoderma viride, Trichoderma harzianum* and *Aspergillus niger* were evaluated against dominant seed borne fungi isolated from barley. It was found that all the biocontrol agents were found significantly effective against *A. alternata, R. stolonifer, Mucor* spp., *F. moniliforme* and *A. flavus*. Among them, *T. viride* was found most effective in percent growth inhibition against seed-borne fungi over control (Table 3).

Biocontrol agents	Percent growth inhibition				
	Alternaria alternata	*Rhizopus* spp.	*Mucor* spp.	*Fusarium moniliformae*	*Aspergillus flavus*
Trichoderma viride	64.14 a	57.26 a	54.64 a	51.60 a	49.40 a
Trichoderma harzianum	58.14 b	52.97 b	49.18 b	46.78 b	44.28 b
Aspergillus niger	46.90 c	44.88 c	42.14 c	41.48 c	38.92 c
C.D. P ≤ 0.05	3.63	3.18	2.72	2.40	2.43

Table 3: Antagonistic activity of bio-agents against seed-borne fungi by dual culture technique.

Each value is the average of three replicates

*Values in a column followed by the different letters are significantly different at P ≤ 0.05 using Duncan's multiple range test

*C.D: Critical Difference

Isolated Fungi	Standard blotter method			Agar plate method		
	Relative frequency			Relative frequency		
	Control	Mancozeb + Carbendazim (300 ppm)	*T.viride* (10^6 conidia/ml)	Control	Mancozeb + carbendazim (300 ppm)	*T. viride* (10^6 conidia/ml)
Alternaria alternata	65.50 a	15.6 a	17.30 a	41.50 a	12.50 a	14.60 a
Fusarium moniliforme	41.80 d	09.15 c	10.60 c	30.75 c	08.30 c	09.50 c
Aspergillus flavus	34.65 e	11.30 b	12.10 b	16.80 f	09.60 b	11.20 b
Aspergillus niger	31.50 e	06.85 d	07.50 d	36.25 b	04.15 d	05.30 d
Penicillium spp.	27.03 f	03.12 e	04.30 e	12.45 g	02.00 e	03.10 e
Drechslera australiensis	18.50 g	02.06 f	03.50 f	27.51 d	01.30 f	02.60 f
Rhizopus Spp.	51.80 B	-	-	24.31 E	-	-
Mucor spp.	47.24 c	-	-	18.40 f	-	-
Curvularia lunata	16.71 g	-	-	24.60 e	-	-
Cladosporium spp.	09.56 h	-	-	-	-	-
Stemphylium spp.	04.51 i	-	-	-	-	-
Ulocladium spp.	02.04 i	-	-	-	-	-
% germination	42	68	64	46	72	70
C.D. P ≤ 0.05	3.40	0.37	0.43	2.68	0.32	0.36

Table 4: Effect of Mancozeb + Carbendazim mixture and Trichoderma viride as seed treatment against seed-borne mycoflora of barley.

Each value is the average of three replicates

*(-) minus sign indicates absence of fungus

*Values in a column followed by the different letters are significantly different at P ≤ 0.05 using Duncan's multiple range test

*C.D: Critical Difference

Fungicidal Seed Treatment

Seeds treatment with Mancozeb + Carbendazim at 300 ppm concentration was found most effective against seed borne fungi. It was found that observed relative frequency of *A. alternata* (15.6%) followed by *F. moniliforme* (9.15%), *A. flavus* (11.3%), *A. niger* (6.85%), *Penicillium* spp. (3.12%) and *D. australiensis* (2.06%) on standard blotter method over control whereas on agar plate method the highest frequency was observed in *A. alternata* (12.5%) followed by *F. moniliforme* (8.3%), *A. flavus* (9.6%), *A. niger* (4.15%), *Penicillium* spp. (2%) and *D. australiensis* (1.3%) as compared to untreated control. The germination percentage of seeds was 68% in standard blotter method and 72% in agar plate method (Table 4).

During pot experiment seed treated with Mancozeb+ Carbendazim mixture at 300 ppm concentration was found significantly effective in increase plant length, fresh weight, dry weight, number of spikelets/spike, number of grains/spike and chlorophyll content over untreated control (Table 5, Figure 1d).

Treatments	Plant Length (cm)	Plant fresh Weight (g)	Plant dry Weight (g)	No. of Spiklets/Spike	No. of rains/Spike	Total Chlorophyll (mg/g)
Control	99.50c	31.93c	10.30c	18.5c	40.5c	1.88c
Mancozeb+Carbendazim (300 ppm)	113.95a	37.76a	16.54a	23.1a	46.51a	2.18a
T. viride (10^6 conidia/ml)	108.65b	34.12b	13.93b	21.85b	43.3b	2.01b
C.D. P ≤ 0.05	4.89	2.11	1.20	1.12	2.14	0.10

Table 5: Effect of Mancozeb+ Carbendazim mixture and Trichoderma viride as seed treatment on the growth and chlorophyll content of barley.

Each value is the average of three replicates

*Values in a column followed by the different letters are significantly different at P≤0.05 using Duncan's multiple range test

*C.D: Critical Difference

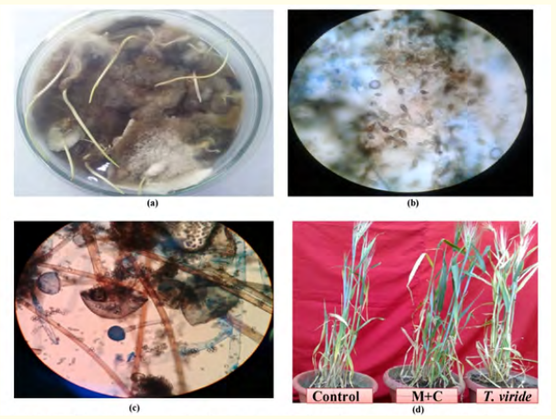

Figure 1: Showa that a. Seed-borne fungi developed on agar media b. *A. alternate* c. *A niger* d. Effect of Mancozeb plus carbendazim mixture (M+C) and *T. viride* as seed treatment on the growth of barley in pot condition.

Seed Biopriming

Seeds treated with *T. viride* showed highest frequency of occurrence in *A. alternata* (17.3%) followed by *F. moniliforme* (10.6%), *A. flavus* (12.1%), *A. niger* (7.5%), *Penicillium* spp. (4.3%) and *D. australiensis* (3.5%) on standard blotter method over untreated control similarly, on agar plate method highest frequency was observed in *A. alternata* (14.6%) followed by *F. moniliforme* (9.5%), *A. flavus* (11.2%), *A. niger* (5.3%), *Penicillium* spp. (3.1%) and D. australiensis (2.6%) as compared to untreated control. The germination percentage of seeds treated with *T. viride* was 64% in standard blotter method and 70% in agar plate method (Table 4).

During pot study seed treated with *T. viride* was found significantly effective in increase plant length, fresh weight, dry weight, number of spikelets/spike, number of grains/spike and chlorophyll content over control plants (Table 5).

Discussion

Standard blotter and Agar plate method were recommended by ISTA for the isolation and detection of seed-borne mycoflora [19]. No single method is adequate in the field of seed pathology for detecting seed-borne fungi associated with seeds [20,21]. Isolation and identification of seed-borne fungi from barley seeds were done through both standard blotter and agar plate methods [22]. It was found that seed-borne fungi from the seeds may be destructive during germination of seeds or may be bringing about mortality soon after the emergence of seedlings, so it is desirable that seeds should be tested for seed health before planting. Among seed-borne fungi *A. alternata* was found most frequent isolated fungi during incubation [23]. Management of seed-borne fungi was done to test the efficacy of fungicides and biocontrol agents *in vitro* [24]. It was found that colony growth of this fungus inhibited significantly in both appliances. Further, application of best dose found to be effective in vitro study as well as pot condition applied as a seed treatment. Application of seed protectants such as fungicides and bioagents helps in producing better emergence and vigorous seedlings [25]. The application of chemicals to the seed is a common and effective means of controlling the majority of seed-borne pathogens. The application of fungicide as seed treatment is the most conventional followed disease control practice used in all crops [26]. Seed treatment with fungicides used against seed-borne mycoflora in many crops [27,28]. Different seed dressing fungicides were applied to bring down the seed-borne inoculum to lowest infestation level and also to see its impact on the germination of seeds [29]. There are a number of modes of action employed by these microorganisms that lead to the seed and seedling protection [30]. Each of these modes of actions has advantages and disadvantages that affect performance. Therefore, mixtures of bio-organisms with different modes of action or combinations of chemicals and biological control agents enhanced the activity but there are limited knowledge and understanding of the interaction of such mixtures. Biological control agents used as seed treatments are being developed by a number of companies across the globe. These products may provide a good solution for seed health and maintain organic status of the crop. It should be stressed that the efficacy of biological seed treatment is at the current time far from reaching the effect of chemical seed treatment; nevertheless, biological treatment can represent on some occasions an interesting complement of chemical protection. So as to avoid the introduction of pathogens in new areas and be treated with nontoxic pesticides like biocontrol agents so as to reduce the pollutant load of the environment.

Conclusion

In dry seed inspection conidial fructifications and abnormalities were observed through unaided eye and stereo-binocular microscope. Seed health techniques showed that highly frequent fungus was *Alternaria alternata* followed by *Rhizopus stolonifer, Mucor* spp., *Fusarium moniliforme* and *Aspergillus flavus* in both the incubation methods. Management of dominant seed-borne fungi was done by Mancozeb, Carbendazim and Mancozeb plus Carbendazim mixture through poison food and dual culture technique respectively. Mancozeb and Carbendazim mixture were caused highest growth inhibition as compared to Mancozeb and Carbendazim alone. In case of bio-agents highest growth inhibition caused by *T. viride* over *T. harzianum* and *A. niger*. Further seed priming with Mancozeb plus Carbendazim and *T. viride* were effective in eliminating majority of seed fungi and percent germination increase in both agar plate method and blotter method. Pot studies shows seed treatment with Mancozeb plus Carbendazim and *T. viride* increases the plant growth and chlorophyll content.

Acknowledgements

The author thanks to the chairman, Department of Botany, Aligarh Muslim University, Aligarh for providing facilities.

Bibliography

1. Rabie CJ., *et al.* "Enumeration of fungi in barley". *International journal of Food Microbiology* 35.2 (1997): 117-127.

2. Bateman GL and Kwasna H. "Effects of number of winter wheat crops grown successively on fungal communities on wheat roots". *Applied Soil Ecology* 13.3 (1999): 271-282.

3. Khanzada KA., *et al.* "Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat". *Asian Journal of Plant Science* 1.4 (2002): 441-444.

4. Agrios GN., "Plant Pathology". 4th edition. Academic Press, California (1997): 245-269.

5. Kadege E and Lyimo HJF. "Prevalence and control of wheat (*Triticum aestivum* L.) seed borne fungi in farmer-saved seeds". *Archives of Phytopathology Plant Protection* 48.7 (2015): 601-610.

6. Harris CA., *et al.* "Assessing the risk of pesticide residues to consumers: recent and future developments". *Food Additives Contaminants* 18.12 (2001): 1124-1129.

7. Annonymous. "Agricultural Statistics of Pakistan Ministry of Food, Agriculture and Livestock". Economic Wing, Govt. of Pakistan Islamabad (2005): 126.

8. Chandler J. "Cost reduction in SIT programmes using auto-dissemination as part of area wide integrated pest management". *International Journal of Pest control* 42.2 (2005): 257-260.

9. Reddy PP. "Biopriming of seeds". *Recent Advances in Crop Protection* (2012): 83-90.

10. Pawar K., *et al*. "Efficacy of bioagents and fungicides against seed borne fungi of soybean". *Annals of Plant and Soil Research* 17.1 (2015): 77-81.

11. Annonymous. "International rules for seed testing". *Proceedings of the International Seed Testing Association* 31(1966): 1-152.

12. Doyer LC. "Manual for the determination of seed-borne diseases". Wageningen, Netherland: International Seed Testing Association (1938): 59.

13. De Temp J. "The blotter method of seed health testing programme". International Seed Testing Association (1953): 133-151.

14. Warham EJ., *et al*. "Seed testing of maize and wheat: A Laboratory Guide". CYMMYT, CAB International, UK (1996): 84.

15. Adams PB and Wong JAL. "The effect of chemical pesticides on the infection of sclerotia of Sclerotinia minor by the biocontrol agent Sporidesmium sclerotivorum". *Phytopathology* 81.10 (1991): 1340-1343.

16. Vincent JM. "Distortion of fungal hyphae in the presence of certain inhibitors". *Nature* 159.4015 (1947): 850-850.

17. Huang HC and Hoes JA. "Penetration and infection of Sclerotinia sclerotiorum by Coniothyrium minitans". *Canadian Journal of Botany* 54.5-6 (1976): 406-410.

18. Mackinney G. "Absorption of light by chlorophyll solutions". *Journal of Biological Chemistry* 140 (1941): 315-322.

19. Malone JP and Musket AE. "Seed-borne fungi, description of 77 fungus Species". *Proceedings of International Seed Testing Association* 29 (1964): l79-384.

20. Neergaard P and Saad A. "Seed health testing of rice: a contribution to development of laboratory routine test methods". *Indian Phytopathology* 15 (1962): 85-111.

21. Christensen CM and Lopez F. "Pathology of stored seeds". Proceedings of International Seed Testing Association 28: 701-711.

22. Ahmad L., *et al*. "Antifungal potential of plant extracts against seed-borne fungi isolated from barley seeds (Hordeum vulgare L.)". *Journal of Plant Pathology and Microbiology* 7 (2016): 350.

23. Levic J., *et al*. "Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in Serbia". *Pesticides and Phytomedicine (Belgrade)* 27.1 (2012): 33-40.

24. Tapwal A., *et al*. "*In vitro* evaluation of Trichoderma Species against seed-borne pathogens". *International Journal of Biological and Chemical Sciences* 1.10 (2015): 14-19.

25. Mancini V and Romanazzi G. "Seed treatments to control seed-borne fungal pathogens of vegetable crops". *Pest Management Science* 70.6 (2014): 860-868.

26. Nene YL and Thapliyal PL. "Fungicides in plant disease control". Oxford and IBH: New Delhi (1979): 413-414.

27. Agarwal GP., *et al*. "Studies on the wheat grain storage in Madhya Pardesh: efficacy of certain fungicides against black-point disease of wheat". *National Academy Science Letters* 4 (1981): 115-117.

28. Raut JG., *et al*. "Seed-borne infection of Alternaria triticina in wheat and its control". *Indian Phytopathology* 36 (1983): 274-277.

29. Habib A., *et al*. "Evaluation of some fungicides against seed-borne mycoflora of eggplant and their comparative efficacy regarding seed germination". *International Journal of Agriculture and Biology* 9.3 (2007): 519-520.

30. Singh S., *et al*. "Antagonistic behaviour of different bio-agents against dominant seed-borne fungi of mungbean seeds under in vitro condition". *Proceedings of National Academy of Sciences India, Section Biological Sciences* 87.2 (2017): 599-602.

Volume 2 Issue 6 June 2018

© All rights are reserved by Lukman Ahamad and Razia K Zaidi.